Shifting Central Tolerance In Type 1 Diabetes

Tolerance Induction By Presenting Peptides In Non-Obese Diabetic Mice

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Introduction

Type 1 Diabetes

Type one diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β-cells in charge of insulin production. The current therapy for T1D allows living with the disease but neither preventing nor reverting it or its long-term complications. Moreover, this treatment leads to suffer and high economic costs for both patients and society, taking into account that it must be taken for life. These facts added to that gene therapy and stem cells will likely take some more years to reach patients, make prevention of T1D by immunological strategies a needing in diabetes research.

NOD mouse

The NOD (Non-Obese Diabetic) mouse has been extensively used in research as a polygenic model of human T1D because it develops spontaneous autoimmunity. It is of relevance that the I-A^{g7} MHC (Major Histocompatibility Complex) haplotype of NOD allele shares high homology with the human T1D susceptibility HLA-DQB1 locus.

Insulin autoantigens: differential presentation between thymus and periphery

Islet APCs are central during autoimmunity, they uptake and present antigens from β-cells, and even can directly uptake secretory granules and their products from β cells. Insulin has been pointed by numerous studies as the main autoantigen in diabetes, and especially the register 9-23 of the B chain (SHLVEALYLVCGERG), whose peptides are presented by I-A^{g7}. Two types of diabetogenic insulin autoreactive T cells were identified: type A and type B. Type B are the most abundant autoreactive T cells in diabetes and they recognize residues 12-20 (B:12-20), meanwhile type A recognize B:13-21. The reason of Type B abundance is that B:12-20 is only presented in Islet APCs by an unconventional pathway and it does not in thymus, this fact allows their bypass of negative selection and consequently their activation in periphery, thereby triggering autoimmunity.

Hypothesis and Objectives

The hypothesis of this project is that the presentation of B:12-20 peptides in thymus deletes by negative selection the most abundant autoreactive T cells contributing to diabetes autoimmunity, type B T cells, and also stimulates the generation of nTregs (Natural Regulatory T cells) specific for B:12-20. Thereby, it diminishes the prevalence of diabetes in Transgenic NOD mice presenting B:12-20 in thymus in comparison to the NOD control ones. The main objectives of this present assay are to:

1. Develop a transgenic NOD mouse which presents B:12-20 peptide in thymus.
2. Study the changes in central tolerance and especially the bypass of autoreactive T cells from thymus due to B:12-20 presentation in Transgenic NOD mice in comparison to control NOD mice.
3. Analyze the effects of B:12-20 peptide thymic presentation on the prevalence, onset, phenotype and immunological features of autoimmune diabetes in Transgenic NOD mice in comparison to control NOD mice.
4. Confirm all the previous studies pointing B:12-20 as one of the main antigens involved in autoimmune diabetes in NOD mouse.

Transgenic Production

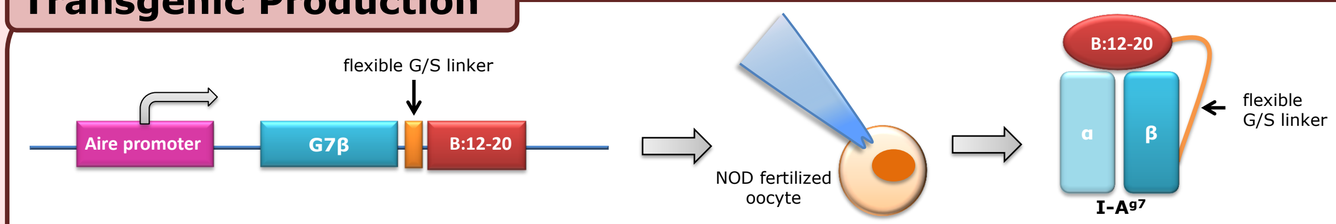

Figure 1. Transgene introduced in NOD mice and its protein product. The transgene will contain a B:12-20 register (VEALYLVCG) covalently bound to the I-A^{g7} β chain (G7β) via a flexible glycine/serine linker (GGGGSLVPRGSGGGGS). The transgene will be under control of *Aire* promoter, thus its expression will be confined only to mTECs (Medullary Thymic Epithelial Cells). Transgenic egg will be produced by microinjection of the transgene cDNA construct into NOD fertilized oocytes. This chimeric protein will join alpha chain to form the peptide-MHC complex (pMHC) within the early vesicles of mTECs and will present B:12-20 peptide in thymus.

Expected Results

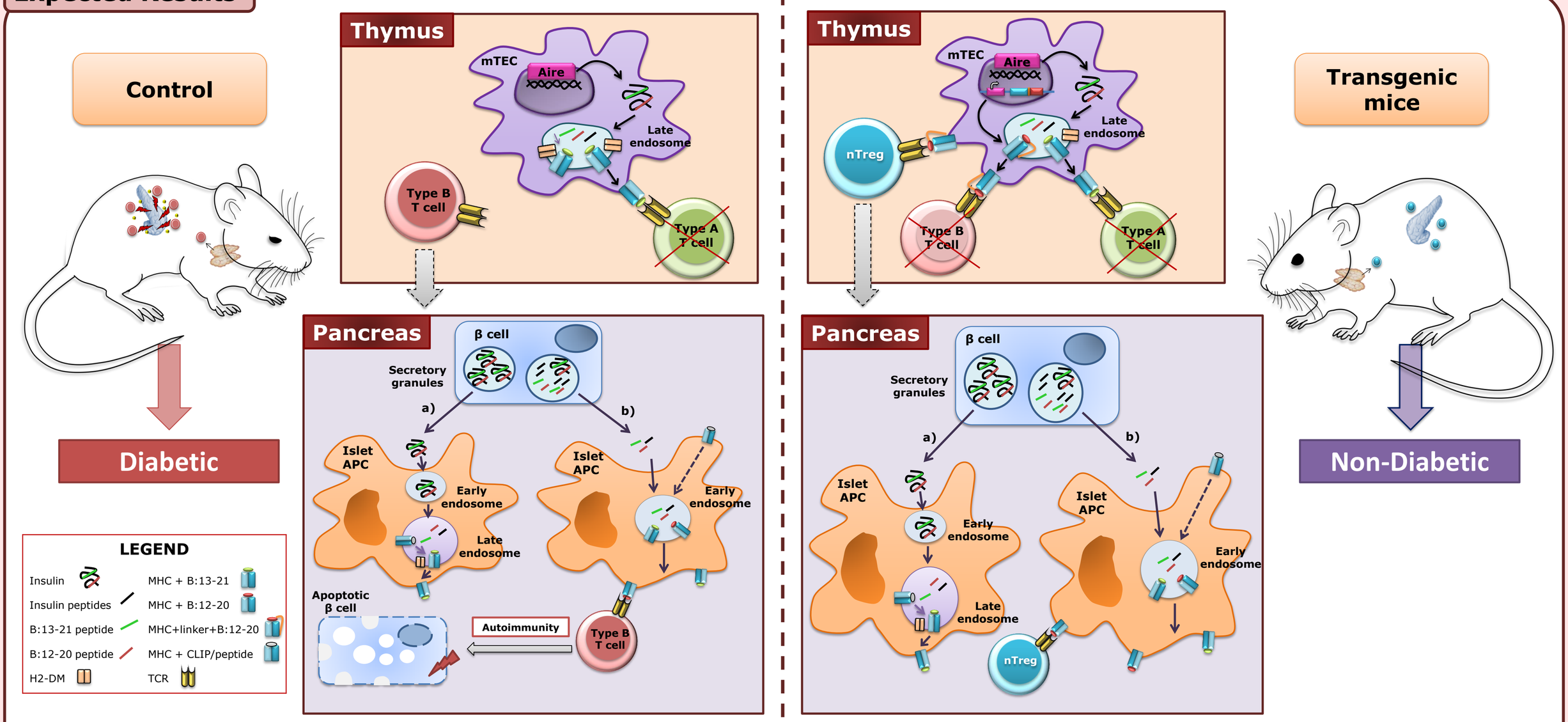

Figure 2. Insulin presentation events in NOD mice and Transgenic NOD mice. In NOD mice insulin is presented by two pathways in Islet APCs in pancreas, in the conventional one (a) insulin is internalized and catabolized into small peptides which in late endosome are exchanged for CLIP and bound to MHC-II if they are specific enough. This process is facilitated by H2-DM chaperone which rapidly eliminate B:12-20 peptides because their weak affinity to I-A^{g7} MHC, thus they cannot be presented. Nevertheless, B:13-21 is well presented due to its high affinity for I-A^{g7}. On the other hand, during the unconventional presentation (b) Islet APCs can take up free peptides from β-cells secretory granules such as B:12-20, these free peptides bind by peptide exchange with MHC-II on plasma membrane, early endosomes or in recycling vesicles where H2-DM is not present. This gives rise to unique insulin peptide-MHC complexes that select for autoreactive T cells, especially type B. Remarkably, the unconventional presentation does not take place in mTEC (Medullary Thymic Epithelial Cells) but only in periphery, so autoreactive T cells specific for B:12-20 (Type B T cells) escape from negative selection, while Type A do not, because B:13-21 is presented in thymus while B:12-20 do not. Therefore, Type B T cells can lead to autoimmunity in pancreas. The expression of autologous B:12-20 pMHC in thymus will avoid or reduce the presence of autoreactive type B T cells in periphery by negative selection and also will stimulate the generation of nTregs specific for B:12-20. Hence, once in pancreas islets, the absence of Type B T cells plus the presence of specific nTreg will prevent the triggering of the autoimmune response.

Experimental Methodology

- **Transgene expression assessment:** Expression will be assessed by pMHC recognition by 8F10 type B T cells, firstly in *in vitro* in mTEC culture under either an ubiquitous promoter and *Aire* promoter; later i*n vivo* studies will be done by thymectomy and subsequent transgenic mTEC culture.
- **Disease detection:** Diabetes detection will be performed by daily urinalysis with reagent strips and confirmation by glucose serum levels >250 mg/dL. Sialitis, other pathologies and general immunosuppression will be checked too.
- **Histology**: Paraffin-embedded tissue sections of pancreas will be stained with hematoxylin and eosin in order to assess insulitis.
- **T cell populations:** Assessment of number of autoreactive type B T cells and nTregs specific for B:12-20 in serum, thymus, pancreatic islets, pancreatic lymph node and spleen will be done with ELISPOT. T cells will be challenged with tetramers bearing B:12-20 pMHC.
- **Autoantibodies levels:** Measurement of levels of antibodies in plasma against insulin epitopes will be determined by ELISA.
- **Adoptive transfer to NOD mice**: Transgenic NOD mice splenocytes will be transferred into NOD mice equally analyzed and treated.

Methods

- The background of this project was extracted from immunology books, reviews and original papers searched on PubMed Database, Science Direct and E-journals database from UAB.
- Some keywords used were "Type 1 Diabetes", "NOD mouse", "Insulin autoantigen", "Diabetes peptides", "Central tolerance diabetes", "Transgenic NOD" and a combination of them. Papers were selected depending on its Journal impact factor and date of publication.
- Hypothesis and objectives were formulated in basis of this information.
- Methodology, techniques and procedures of the project were determined by previous knowledge and by previous similar studies.